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CHOLINERGIC DRUG EFFECTS ON REACTION SPEED AND SHORT-TERM MEMORY OF PRIMATES

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NOTICES

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The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Animal Resources - National Research Council.

The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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The effects of four cholinergic drugs on the speed and accuracy of performance of a short-term memory (STM) task by rhesus monkeys were measured. The drugs and doses studied were atropine sulfate and atropine methylnitrate (0.044, 0.140, and 0.440 mg/kg), physostigmine sulfate (0.05, 0.10, and 0.15 mg/kg), pyridostigmine bromide (0.15, 0.20, and 0.25 mg/kg), and all possible combination doses of atropine sulfate (0.0, 0.14, and 0.44 mg/kg) and physostigmine sulfate (0.0, 0.075, and 0.10 mg/kg). The STM task was a three-alternative delayed match-to-sample paradigm with hue the relevant stimulus dimension and delay (retention interval) titrated so as to maintain performance at approximately 75% correct. The following performance measures were examined: median retention interval (the measure of STM), speed of response to sample stimulus, speed of response to match stimuli when the correct match was chosen, and speed of response to match stimuli when the incorrect match was chosen.

There were no significant effects of any drug on STM, although there were suggestions that physostigmine can facilitate and atropine can interfere with STM. These suggestive

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findings are in agreement with results from other laboratories. At high doses, physostigmine completely disrupted performance of the STM task. Atropine reversed this effect.

In contrast to STM, response speed was quite sensitive to the effects of centrally active cholinergic drugs. Both atropine sulfate and physostigmine sulfate alone produced decrements in all three response speed measures. However, these two drugs tended to antagonize one another's effects, so that the response speed decrement produced by one could be cancelled by an appropriate dose of the other.

The three types of response measured were found to differ reliably in speed. Responses to the sample stimuli were faster than correct match responses, which, in turn, were faster than incorrect match responses. Thus, response speed could be used as an index of the probability of an operator error, in some contexts.

CHOLINERGIC DRUG EFFECTS ON REACTION SPEED AND SHORT-TERM MEMORY OF PRIMATES

INTRODUCTION

The successful performance of many U. S. Air Force tasks depends on the ability to respond rapidly and accurately to incoming information. Because the correct response often depends on the preceding sequence of events, short-term memory (STM), the ability to retain and act upon information for periods of seconds to minutes, is often critical for accurate response. To the extent that STM involves cholinergic neural processes, it is vulnerable to the effects of chemical warfare agents, antidotes and pretreatment drugs that modify cholinergic processes. Thus, it is important to assess the effects of chemical defense compounds on the performance of tasks that require STM. Since drugs may affect either the speed or the accuracy of response, or both, it is important to measure both components of task performance.

Three experiments on the effects of cholinergic drugs on speed and accuracy of STM task performance by laboratory primates are reported here:

1) The first experiment compared the effects of two carbamate anticholinesterases, pyridostigmine and physostigmine. Pyridostigmine is a fielded chemical defense pretreatment drug that penetrates the blood-brain barrier to a small extent at most. Physostigmine, which penetrates the blood-brain barrier to a much greater extent, has been proposed as a pretreatment drug. It is also of interest because it may mimic some of the effects of anticholinesterase chemical warfare agents.

2) The second experiment compared the effects of two anticholinergic drugs, atropine sulfate and atropine methylnitrate. Atropine sulfate is a component of all fielded chemical defense antidotes. It penetrates the blood-brain barrier readily. Atropine methylnitrate, like pyridostigmine, is a quaternary compound that penetrates the blood-brain barrier to a much smaller extent.

3) The third experiment investigated the performance effects of combinations of atropine sulfate and physostigmine. These two centrally active drugs have antagonistic effects on cholinergic systems. The extent to which they might cancel each other's effects on the accuracy and speed of STM task performance has not been investigated previously.

The effects of cholinergic drugs on memory have been the subject of a number of investigations (1-4, 6, 9, 11-13, 19, 20, 22, and 24). The present experiments differ from previous ones in three respects: 1) A larger array of drugs and doses was studied. 2) The method used to measure STM in these experiments

involved a titration procedure not commonly used. 3) Speed of reaction to all stimuli was measured, providing an opportunity to examine the effects of chemical defense drugs on reaction speed in a "choice" context, as well as in a simple reaction-time task.

The drugs and doses used, as well as relevant prior studies, will be presented in the context of the individual experiments. The general methods for STM and reaction speed measurement were the same for all three experiments. Methods and procedures common to all three experiments will be presented before the individual experiments are discussed.

GENERAL METHODS AND PROCEDURES

Measurement of STM

The task most commonly used to measure STM in laboratory animals is the delayed match-to-sample (DMTS) task. At the beginning of each trial, the sample, one of a set of stimuli differing in one or more dimensions (e.g., hue, size, or shape) is presented to the animal. After the animal has made a response indicating attention to the sample, this stimulus is removed, and a delay (the retention interval) ensues. At the end of the retention interval, several stimuli are presented, only one of which is a "match" to the sample. The animal's task is to remember which sample was presented, and to choose the "match" from among the alternatives presented after the delay.

Two strategies have been used to measure differences in the ability of animals to remember as a function of differences in drug doses. One is to present a long series of trials in which the members of a specified set of retention intervals are tested many times in a randomized order, so that the probability of a correct recall at each retention interval can be estimated. This kind of series is repeated for each dose. As Bartus and Johnson (4) have pointed out, to demonstrate a drug effect on retention (STM), it is necessary to measure a significant interaction between dose and retention interval, such that the probability of a correct match is more affected at longer retention intervals by higher doses. This method has two disadvantages:

a) Very large numbers of test trials are required.
b) Dose effects on memory are confounded with extinction effects (e.g., frustration, lack of incentive).

The first disadvantage arises from the range of retention intervals that must be sampled (long intervals to show weak effects -- short ones to demonstrate strong effects when the animal cannot perform at longer intervals). The second disadvantage arises from the fact that success rates necessarily vary with dose. At doses that have strong effects, the animals' ability to perform correctly (especially at long retention intervals) may fall to the extent that they have no incentive to "work at" the task, since no amount of effort can raise their performance (and reward) above a chance level.

The titration method, the second commonly used strategy for measuring drug effects on STM, reduces both of these problems by minimizing the number of trials necessary to assess memory capacity and by holding success rates approximately constant (16). The essential feature of the titration method is to make the retention interval contingent on the performance of the animal. When STM is impaired, so that the animal cannot perform well at long intervals, the retention interval is adjusted downward, and vice versa. Over a series of trials, the average retention interval is a direct measure of STM ability.

Reaction Speed Measurement

In addition to the average retention interval, all response latencies were recorded. Several processes are involved in performing the complex DMTS task. First, the animal must attend to the sample presentation. The latency of response to this presentation (or its inverse, the response speed) provides an index of attention. After the retention interval, the animal must choose among the alternatives presented. The speed of the choice response reflects both attention to the task and the cognitive processes involved in choosing. Similar experiments (5, 16, 21) have shown that speed of response to the sample is reliably faster than to the match stimuli, and that unsuccessful match responses (errors) are slower than correct match responses. Since the effects of cholinergic drugs on attention and choice processes might prove to be as important as their effects on STM, the response speed data were also analyzed.

Subjects

The subjects were six 4- to 6-year-old male rhesus monkeys (Macaca mulatta) with 6 months to 1 yr of experience with the delayed match-to-sample task. They were maintained on a 12:12 h light/dark cycle with lights on at 0400. They were fed twice daily and water deprived for 16 h prior to testing.

Apparatus

The monkeys were tested in sound attenuating chambers (Industrial Acoustics Company), in the presence of a masking white noise and an overhead house light (1A, 28V). Four in-line digital display units (Industrial Electronic Engineering, Inc.) were mounted behind a panel with a circular hole (2.54 cm dia) for each display unit. The displays were configured with 3 above and 1 below. The 3 above were 7 cm apart (center-to-center) and 9 cm each from the center of the display below. Each display was recessed 1.4 cm behind the panel to allow for a photodetection circuit in front of each display. These circuits provided a response input each time a monkey touched one of the displays. Each chamber was equipped with a delivery tube for liquid reward. The volume of each reward (approximately 0.1 mL) was determined by a solenoid valve (LSC-001, BRS Foringer). The apparatus was

controlled by a laboratory computer that programmed all stimulus-response contingencies and recorded the data (8).

Procedure

Appendix A describes the contingencies in the titration procedure in detail. The Appendix also includes a flow diagram (Fig. A-1) that shows how the sequence of events during a test session was determined, as well as the sources of the data. One of four hues was randomly selected as the sample stimulus for each trial. The position of the correct match presentation varied randomly from trial to trial, as did the hues and positions of the incorrect matches. Each test session began with a brief warm-up period, during which no data were collected. The retention interval for the first warm-up trial was set at 2 s. The retention interval was increased by 2 s for each correct match until the warm-up period ended, either when a correct match occurred at a delay interval of 10 s, or when 2 min had elapsed, whichever came first. Data collection began with the retention interval set at 10 s for the first test trial.

The monkeys were tested each weekday between 0400 and 0800 for 30 min after the end of the warm-up. For testing, each animal was secured in a chair in a sound attenuating chamber with the stimulus-response panel at eye level and a drinking tube near his mouth. Each trial began with the onset of a colored light in the lower display (sample stimulus). The monkey indicated attention to the sample stimulus by touching it, at which time the light was extinguished and a juice reward occurred on one-third of the trials. The offset of the sample was followed by a variable retention interval that preceded the onset of lights in the three upper display units (match stimuli). The match stimuli were three different colors, one of which was always the same color as the sample stimulus. A response to a match stimulus was considered correct if the match stimulus was the same color as the immediately preceding sample. Correct responses to the match stimuli were always rewarded with juice followed by a 2 s delay before the next trial began. Incorrect responses resulted in a variable time-out period with the house light off. Inappropriate responses during the retention interval resulted in a time-out period followed by a new trial. The variable retention interval was determined by the match response and retention interval on the previous trial. If the match response on the previous trial was incorrect, the retention interval on the current trial was decreased one step from the retention interval on the previous trial. If the match response was correct, the retention interval was increased by one step with a probability of one-third. The size of the step was 2 s for intervals less than 20 s and 4 s for intervals greater than 20 s. This titration procedure assured that an animal attempting to match-to-sample would be rewarded for correct responses on an average of 75% of the completed trials. The median retention interval for each session was recorded as a general indicator of short-term memory. Latency to respond to the sample, latency to a correct match, and latency to

an incorrect match were recorded and transformed into response speed for each trial by means of a reciprocal transformation, and averaged for each test session.

Drug effects were tested on Tuesdays and Fridays. Injection volumes of drug or saline vehicle were always 0.1 mL/kg of body weight. Injections were intramuscular (lateral aspect of thigh); they were given 30 min prior to behavioral testing.

EXPERIMENT I - EFFECTS OF PYRIDOSTIGMINE AND PHYSOSTIGMINE

The theory that cholinergic systems are intimately involved in normal memory function has received considerable experimental support in recent years. Anticholinergics have been shown to disrupt STM in human and nonhuman primate subjects (e.g., 1, 4, 9, 19). If this disruption effect arises from interference with a cholinergic mechanism directly involved in memory storage, it follows from the theory that drugs that facilitate cholinergic transmission (e.g., anticholinesterases) might, in some instances, improve STM. Such a facilitation effect of carbamate anticholinesterases (physostigmine or neostigmine) has been sought in a number of experiments (1-4, 11, 12, and 19), with mixed results. While an STM facilitation effect has sometimes been found, it appears to be idiosyncratic and not very robust. With physostigmine, facilitation sometimes occurs at low doses (.1 to .2 mg/kg), but a performance decrement develops at higher doses. Penatar and McDonough (19), using a DMTS task with rhesus monkeys, found no effect of physostigmine (.25 - .75 mg/kg) on STM. However, they used the traditional method of sampling retention at four fixed retention intervals, and found that the monkeys simply stopped responding to the task for periods of time that increased with dose. They were, therefore, unable to measure STM during the period when the drug was most likely to affect it.

The present experiment examined the effects of two carbamates on STM and reaction speed. The effects of pyridostigmine are of interest for two reasons: a) pyridostigmine has been fielded as a chemical defense pretreatment drug, so its effects are of operational significance, and b) pyridostigmine penetrates the blood-brain barrier to a much smaller extent than physostigmine, so differences in the effects of the two drugs can provide a basis for inferences as to the locus of the effects (central nervous system or peripheral). The effects of physostigmine are of interest because it is a potential chemical defense pretreatment drug that has been shown to afford greater protection than pyridostigmine (Green, Leadbeater and Reynolds, unpublished data). The present study directly compares the effects of these two carbamates on STM and reaction speed.

Methods and Procedures

The doses of physostigmine sulfate were .0 (control), .050, .075, and .100 mg/kg. The doses of pyridostigmine bromide were .0 (control), .150, .200, and .250 mg/kg. Physiological saline (.9%) was the drug diluent and the control injection. Corresponding doses of pyridostigmine and physostigmine were expected to produce approximately equal inhibition of serum cholinesterase, based on prior experience in our laboratory (10; Bennett, unpublished data). Every animal received each dose of each drug on two occasions, so replication was included in the experimental design. Order of dose administration was randomized for each replication for each animal. Median retention interval, sample response speed, correct match response speed, and error response speed were the dependent variables. To determine whether either drug induced long pauses in task performance, the number of trials completed during the first 15 min of the test sessions were counted and analyzed.

Results

The data were analyzed by repeated-measures analysis of variance (ANOVA) with three within-subjects factors: drug, dose, and replication. The results of all ANOVAs performed are tabulated in Appendix B. A separate analysis was performed for each dependent variable: median retention interval, mean speed of response to sample stimulus (sample speed), mean response speed for correct matches (correct speed), and mean speed of error matches (error speed).

The analysis of retention interval showed no reliable effects of the drugs on STM. However, response speed was affected differentially by physostigmine and pyridostigmine (Fig. 1).

Data for sample speed, correct speed, and error speed are shown as Parts A, B, and C, respectively, in Figure 1 and subsequent figures in which response speed is the dependent variable. For all three classes of response, response speed remained near control levels for all doses of pyridostigmine, but tended to decline progressively with increasing doses of physostigmine. This tendency was reflected in a significant ($p < .05$) drug by dose interaction for correct speed (Fig. 1B) and a nearly significant ($p < .06$) drug by dose interaction for error speed (Fig. 1C).

As Penatar and McDonough (19) observed, there was a tendency for animals to quit performing the task under the influence of physostigmine. We analyzed this tendency with a repeated measures ANOVA of the number of trials completed within the first 15 min of testing (dose and replications were the independent variables). The significant ($p < .05$) dose effect is shown in Figure 2. A similar analysis for the effect of pyridostigmine on trial completion showed no significant effects or interactions.

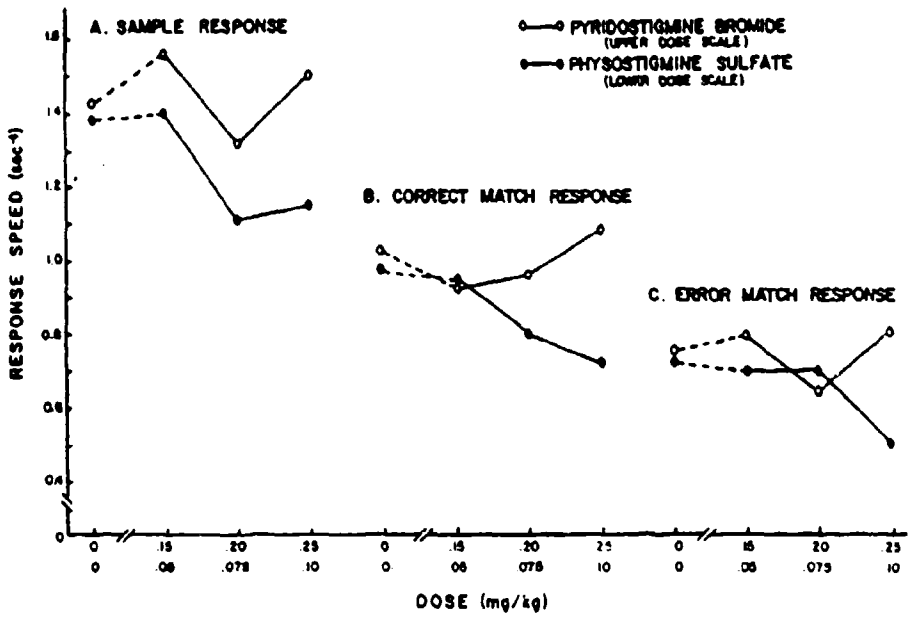

Figure 1. Mean speeds of responses in the DMTS task as a function of pyridostigmine bromide and physostigmine sulfate doses.

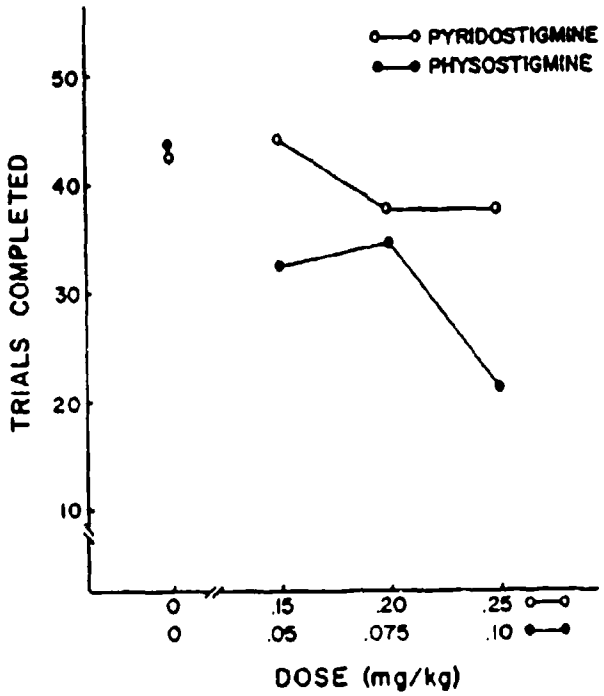

Figure 2. Mean number of DMTS trials completed in 15 min of testing under the influence of physostigmine sulfate or pyridostigmine bromide.

Discussion

Neither pyridostigmine nor physostigmine produced reliable changes in STM. Physostigmine produced a dose-related depression of response speed. The drug by dose interaction shown in Figure 1B illustrates this effect most clearly. The two highest doses of physostigmine reduced response speed 15-25%. However, pyridostigmine, at doses about twice as great as those required for protection against soman lethality (McDonough, unpublished data) produced no reliable changes in STM or reaction speed. Physostigmine also caused the animals to stop performing the task for variable periods of time during the session, while pyridostigmine had no such effect. These findings suggest that the use of physostigmine as a chemical defense pretreatment drug might jeopardize the performance of tasks that require quick reactions to incoming information, while pyridostigmine is relatively safe for such prophylactic treatment.

EXPERIMENT II - ATROPINE SULFATE VS ATROPINE METHYLNITRATE

Anticholinergic drugs (e.g., atropine and scopolamine) interfere with neural and neuromuscular processes by blocking (reducing) the action of the transmitter substance acetylcholine. While atropine is the antidote of choice for anticholinesterase intoxication, its effects on performance of STM tasks have been studied very little. However, there has been considerable research on the effects of scopolamine, a similarly acting cholinergic. In 1967, Bohdanecky and his colleagues (9) showed a dose-related deficit in STM in laboratory primates for scopolamine, but not methylscopolamine. Since methylscopolamine has actions similar to scopolamine at peripheral neural and neuromuscular synapses, but penetrates the blood-brain barrier poorly, Bohdanecky et al. concluded that the deleterious effects of scopolamine on STM were due to its effects on the central nervous system. In a 1971 review of literature on the effects of scopolamine, Safer and Allen (22) concluded that it impairs human STM. More recent studies (20, 24) have further delineated the deleterious effects of scopolamine on human learning and memory. Extensive experimental work in rodents (6, 13) and laboratory primates (4) has shown that the human STM deficits induced by scopolamine are well modeled in laboratory animals.

The effects of atropine on STM are not as well documented as those of scopolamine. Drug discrimination studies (15, 18) have shown that atropine's effects on the nervous system are closely related to those of scopolamine. Roberts and Bradley (21) examined the effects of atropine sulfate and atropine methylnitrate on performance of a delayed discrimination task, and found that both drugs disrupted performance. This finding is not consonant with the results for scopolamine and methylscopolamine reported above, and leaves open the question of atropine's effects on central processes involved in STM. Penatar and McDonough (19) have demonstrated dose-related deficits in STM in monkeys on a DMTS

task produced by atropine sulfate. However, their experiments did not involve atropine methylnitrate, so the question of central vs peripheral effect remains open.

Methods and Procedures

The doses of atropine sulfate and atropine methylnitrate used were: .0 (control), .044, .140, and .440 mg/kg. Physiological saline (.9%) was used as a drug diluent and control injection. Atropine sulfate and atropine methylnitrate were chosen because of their well known anticholinergic properties and differential penetrance of the blood-brain barrier. Doses were chosen on the basis of previous experience in our laboratory and others (17). Order of dose administration was determined by a random process. Each animal received each drug and dose on two occasions, so the experimental design included replication as a treatment effect. As in Experiment I, the number of trials completed in the first 15 min of each session was recorded and analyzed.

Results

The data were analyzed as in Experiment I. The analysis for the median retention interval data indicated a significant ($p < .05$) replication effect. This effect indicates that the subjects' memory performance improved from the first (10.07 s) to the second replication (12.58 s). Neither drug nor dose affected retention interval. The analysis of the sample speed data indicated a significant ($p < .01$) dose effect (Fig. 3A). As dose increased, response speed decreased, regardless of which drug was administered. The analysis of correct response speed data (Fig. 3B) did not provide any statistically reliable effects or interactions although the drug effect approached significance ($p < .07$).

The analysis of error speed indicated a significant ($p < .05$) dose effect and a significant ($p < .05$) drug by dose interaction (Fig. 3C). For atropine, error response speed generally decreased as dose increased whereas for methylatropine, the error response speed did not change.

Neither atropine sulfate nor atropine methylnitrate had reliable effects on the number of trials completed by the subjects.

Discussion

Neither atropine sulfate nor atropine methylnitrate produced reliable changes in STM. The significant replication effect for the median delay data was due to practice effects. The subjects were required to achieve a 70% correct criterion on the titration before exposure to any drug. Approximately 2 months elapsed between the end of the first replication and the beginning of the second replication. During that time, the animals continued training on the task and their performance improved, as

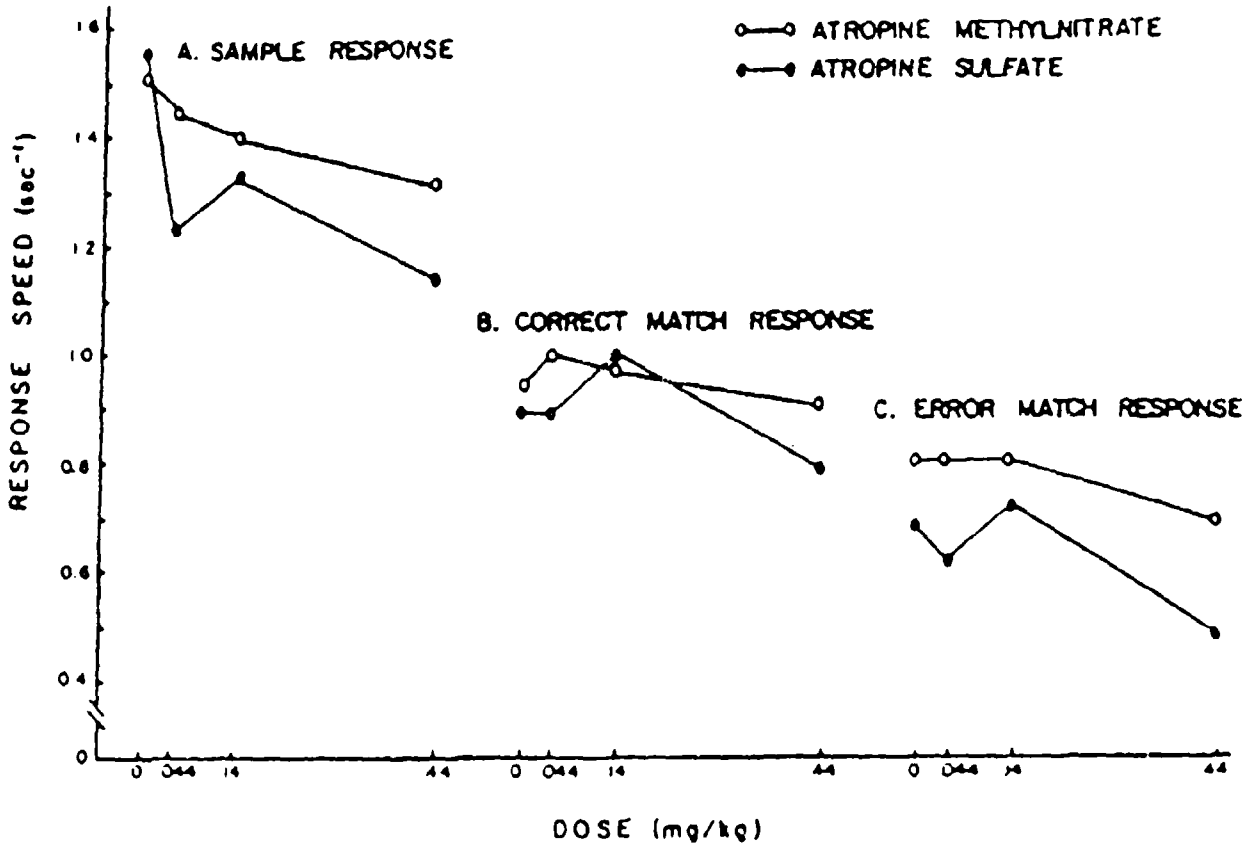

Figure 3. Mean STM retention interval after doses of physostigmine (low = .075 mg/kg, high = .10 mg/kg) or atropine low = .14 mg/kg, high = .44 mg/kg).

indicated by the longer retention intervals in the second replication. Longer retention intervals indicate that the subjects made more correct responses at shorter retention intervals and therefore increased the interval more frequently during the second replication.

The significant dose effect for sample response speed was due to a decrease in speed as dose increased regardless of the drug. Apparently, anticholinergic drug treatment slows sample response speed, which we have taken as indicative of attentiveness. Since both atropine sulfate and atropine methylnitrate produced this effect, we must conclude that the effect arose from peripheral effects of the drugs. This is in agreement with the results of Roberts and Bradley (21), who found depressed matching accuracy with both atropine sulfate and atropine methylnitrate. The effect did not include a drug interaction with retention interval. The neuromuscular junction is a possible site of this peripheral effect since neuromuscular synapses are almost always cholinergic. However, if this hypothesis were true, we would expect a significant slowing of all response speeds, which did not occur. Such slowing did not occur for either correct or incorrect match responses. Other possible peripheral sites of action are numerous. Peripheral actions of the drugs may have produced distracting effects, since this experiment was the first drug-induced alteration of systemic chemistry experienced by these animals.

The significant drug by dose interaction for error response speed is the most interesting of the effects. Figure 3C illustrates that the highest dose of atropine caused a significantly slower error response than either the highest dose of methylatropine or the placebo control ($t(15) = 2.5$, $p < .025$, and $t(15) = 2.4$, $p < .025$ respectively). The same tendency occurred for sample speed and correct match speed, though neither of these effects was statistically reliable. It appears that with doses of atropine low enough to leave short-term memory intact (i.e., no significant dose effect or dose X drug interaction for retention interval), error response speed is significantly reduced. Since errors are clearly associated with conditions of high uncertainty, this implies that the central effects of atropine may be most apparent in a depression of reaction speed under such conditions.

Unlike physostigmine, atropine did not cause long pauses in task performance.

EXPERIMENT III - COMBINED EFFECTS OF ATROPINE AND PHYSOSTIGMINE

Even though the effects of physostigmine and atropine are pharmacologically antagonistic, Experiments I and II showed that both drugs produce a slowing of response speed. Also, physostigmine induced a tendency to quit performing the task, but atropine did not. Experiment III was designed to determine whether these

effects would be potentiated by administering the drugs in combination, or whether the pharmacologic antagonism would lead to a cancellation of effects. This question has clear relevance to chemical defense, since there are many scenarios in which personnel might be exposed to both an anticholinesterase (nerve agent or pretreatment drug) and an anticholinergic (antidote) simultaneously.

Methods and Procedures

Atropine sulfate doses were .0, .14, and .44 mg/kg. Physostigmine sulfate doses were .0, .075, and .1 mg/kg. These drugs and doses were factorially combined. The order of administration of the nine possible combinations was randomized for each monkey.

Results

The data for each of the dependent variables were analyzed by repeated measures factorial analysis of variance. The overall analysis of retention interval indicated no significant STM effects. However, specific comparisons of individual doses of the drugs with control data by t-test indicated a reliable ($p < .05$) decrease in retention interval caused by .44 mg/kg of atropine, and a nearly reliable ($p < .08$) increase caused by .075 mg/kg of physostigmine (Fig. 4). While these effects are only suggestive, they are in agreement with previous findings (2, 4).

The analysis of sample response speed indicated a significant ($p < .001$) interaction between the dose effects of atropine and physostigmine (Fig. 5A). Sample response speed was slowed by either atropine or physostigmine alone, but in combination each drug tended to offset the other's effects. For example, the high dose of physostigmine combined with zero atropine produced a very slow response speed; the same dose of physostigmine combined with the high dose of atropine produced a response speed near control levels. The high dose of physostigmine combined with the low dose of atropine produced an intermediate response speed. This pattern is repeated for varying doses of physostigmine combined with the high dose of atropine.

The analysis of correct response speed also indicated a significant ($p < .001$) atropine-physostigmine interaction (Fig. 5B). Low doses of each drug, separately and combined, reduced correct response speed only moderately. High doses of the two drugs reduced correct response speed most when administered separately; when administered together, high doses of atropine and physostigmine completely antagonized one another's effects on correct response speed.

The analysis of error response speeds produced results similar to those for sample and correct response speeds; the atropine-physostigmine interaction (Fig. 5C) was significant ($p < .001$). As with sample speed, the low dose of atropine was a less

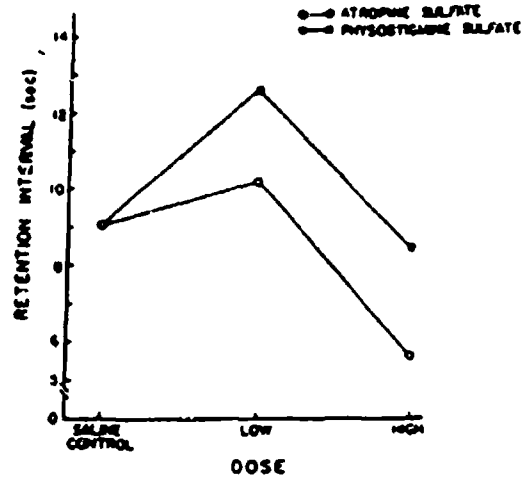

Figure 4. Mean speeds of responses in the DMTS task as a function of atropine methylnitrate and atropine sulfate doses.

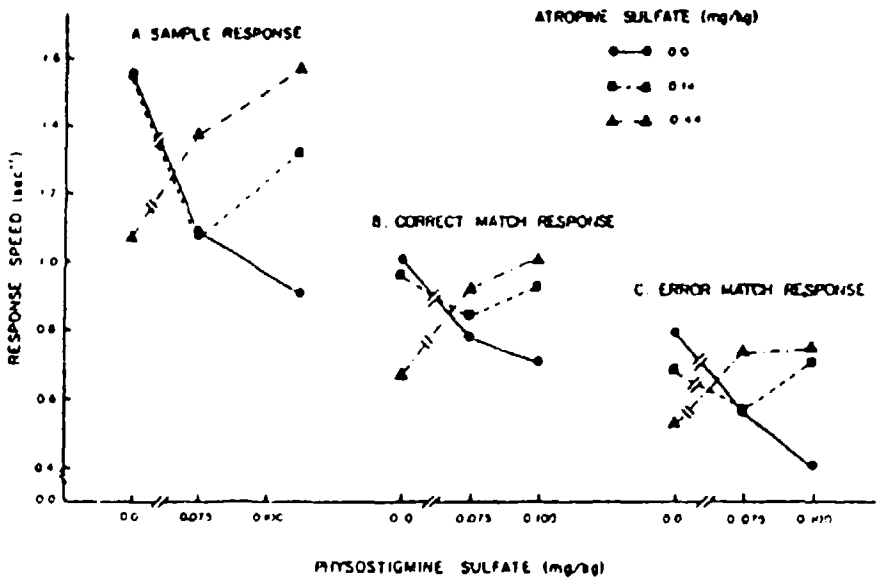

Figure 5. Mean speeds of responses in the DMTS task with combinations of atropine and physostigmine doses.

effective antagonist of the low dose of physostigmine than of the high dose of physostigmine.

As in Experiment I, physostigmine tended to make the animals stop performing the task for variable periods of time. An ANOVA of the number of trials completed in the first 15 min showed a significant ($p < .05$) physostigmine by atropine interaction (Fig. 6). Physostigmine alone reduced the number of trials completed in a dose-related fashion. Atropine did not have this effect, but atropine did reduce the effect produced by physostigmine. In fact, the high dose of atropine completely cancelled the physostigmine effect.

Discussion

The results of Experiment III, like those of Bartus (2), did not indicate a strong facilitative effect of physostigmine on short-term memory (STM). Our low dose of physostigmine did produce a mean retention interval several seconds higher than the placebo. However, this difference was not statistically reliable. Likewise, although the high dose of atropine in the present study produced the lowest mean retention interval, implying an interference with STM like that found by others (19), the effect was only suggestive.

As both Experiment I and Penatar and McDonough (19) showed, physostigmine interfered with the performance of the DMTS task in that animals stopped performing at high doses. At the highest dose, all but one animal in the present study completed substantially fewer trials than normal. Combining atropine with physostigmine reversed the effect.

GENERAL DISCUSSION AND CONCLUSIONS

The drugs that are of highest interest for their prophylactic or antidotal use in response to the threat of nerve agent intoxication, pyridostigmine and atropine respectively, do not have functionally important effects on STM at doses that would be used therapeutically. Since pyridostigmine produced no major effects on memory or on reaction speed at such doses, our results indicate that its use in a prophylactic combination would be relatively safe. Both physostigmine and atropine produced minor effects on STM and more substantial effects on reaction speed, but the results indicate that each of these drugs tends to cancel the effects of the other. The cancellation implies that atropine would tend to ameliorate the effects of low level exposure to nerve agents on STM and on reaction speed. Physostigmine also interfered with performance in that animals tended not to perform the task at the higher doses. Atropine also reversed this effect.

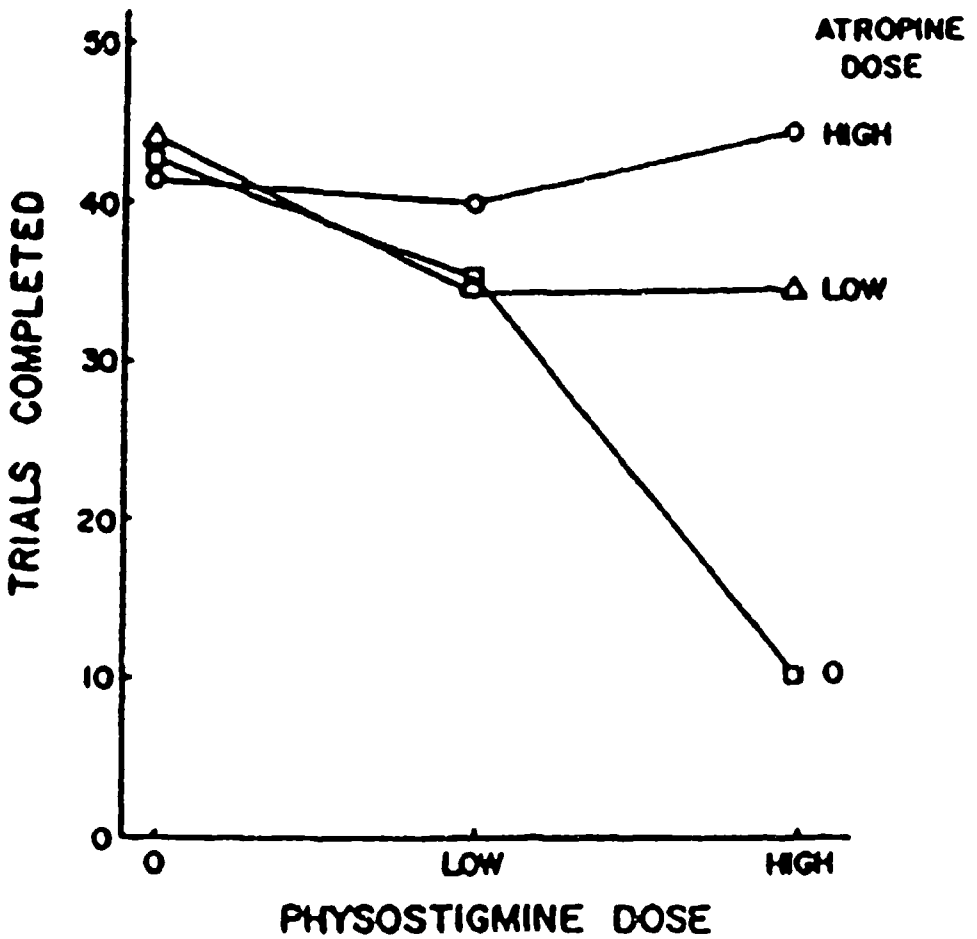

Figure 6. Mean number of DMTS trials completed in 15 minutes after combinations of atropine and physostigmine doses.

Reasoning by analogy from these results, we would predict that a dose of atropine methylnitrate could be found that would cancel any deleterious side-effects of a prophylactic administration of pyridostigmine. Reaction speed appears to be a sensitive indicator of the performance effects of cholinergic compounds of interest in chemical defense research. Reaction speed is slowest when subjects make errors. For some tasks, slow reaction speeds might be so highly predictive of errors that the probability of costly errors could be greatly reduced by structuring the task so that such slow responses have no effect, except to require the operator to repeat the last operation.

REFERENCES

1. Alpern, H. P., and J. G. Marriott. Short-term memory: facilitation and disruption with cholinergic agents. Physiol Behav 11:571- 575 (1973).

2. Bartus, R. J. Physostigmine and recent memory: effects in young and aged nonhuman primates. Science 206:1087-1089 (1979).

3. Bartus, R. J., R. L. Dean, and B. Beer. Memory deficits in aged cebus monkeys and facilitation with central cholinomimetics. Neurobiol Aging 1:145-152 (1980).

4. Bartus, R. J., and H. R. Johnson. Short-term memory in the rhesus monkey: disruption from the anticholinergic scopolamine. Pharmacol Biochem Behav 5:39-46 (1976).

5. Bauer, R. H., and J. M. Fuster. Effects of d-amphetamine and prefrontal cortical cooling on delayed matching-to-sample behavior. Pharmacol Biochem Behav 8:243-249 (1978).

6. Biederman, G. B. The search for the chemistry of memory: recent trends and the logic of investigation in the role of cholinergic and adrenergic transmitters. In G. A. Kerkut and J. W. Phillis (eds.). Progress in Neurobiology, Vol 2, part 1. New York: Pergamon Press, 1975.

7. Birtley, R. R. N., J. B. Roberts, B. H. Thomas, and A. Wilson. Excretion and metabolism of [14C]-pyridostigmine in the rat. Brit J Pharmacol 26:393-402 (1966).

8. Blick, D. W., J. T. Yates, and T. G. Wheeler. MANX: A system for computerized control of and data acquisition from behavioral experiments. USAFSAM-TR-82-6, 1982.

9. Bohdanecky, Z., M. E. Jarvik, and J. L. Carley. Differential impairment of delayed matching-to-sample in monkeys by scopolamine and scopolamine bromide. Psychopharmacology 11:293-299 (1967).

10. Campbell, M. E., D. W. Blick, J. Lanum, T. G. Wheeler, and G. D. Callin. Blood cholinesterase as a function of physostigmine. USAFSAM-TR-81-19, 1981.

11. Davis, K. L., R. C. Mohs, B. M. Davis, G. S. Rosenberg, J. H. Horvath, and Y. DeNigris. Cholinomimetic agents and human memory: preliminary observations in Alzheimer's disease. In G. Pezeu and H. Ladinsky (eds.). Cholinergic Mechanisms. New York: Plenum Press, 1981.

12. Davis, K. L., R. C. Mohs, J. R. Tinklenberg, A. Pfefferbaum, L. E. Hollister, and B. S. Kopell. Physostigmine: improvement of long-term memory processes in normal humans. Science 201:272-274 (1978).

13. Deutsch, J. A. The cholinergic synapse and the site of memory. Science 174:788-794 (1971).

14. Dirnhuber, P., M. C. French, D. M. Green, L. Leadbeater, and J. A. Stratton. The protection of primates against soman poisoning by pretreatment with pyridostigmine. J Pharm Pharmacol 31:295-299 (1979).

15. Hughes, R. N. A review of atropine drug effects on exploratory choice behavior in laboratory rodents. Behav Neural Biol 34:5-41 (1982).

16. Jarrard, L. E., and S. D. Iverson. Recognition, memory, chlordiazepoxide and rhesus monkeys: some problems and results. Behav Brain Res 1:227-243 (1980).

17. McDonough, J. H., Jr. Effects of anticholinergic drugs on DRL performance of rhesus monkeys. Pharmacol Biochem Behav 17:85-90 (1982).

18. Overton, D. A. Discriminable effects of antimuscarinics: dose response and substitution test studies. Pharmacol Biochem Behav 6:659-666 (1977).

19. Penatar, D. M., and J. H. McDonough, Jr. Effects of cholinergic drugs on delayed match-to-sample performance of rhesus monkeys. Pharmacol Biochem Behav 19:963-967 (1983).

20. Petersen, R. C. Scopolamine induced learning failures in man. Psychopharmacology 52:283-289 (1977).

21. Roberts, M. H. J., and P. B. Bradley. Studies on the effects of drugs on performance of a delayed discrimination. Physiol Behav 2:389-397 (1967).

22. Safer, D. J., and R. P. Allen. The central effects of scopolamine in man. Biol Psychiat 3:347-355 (1971).

23. Sahgal, A., and S. D. Iverson. Recognition memory, chlordiazepoxide and rhesus monkeys: some problems and results. Behav Brain Res 1:227-243 (1980).

24. Sitaram, N., H. Weingartner, and J. C. Gillin. Human serial learning: enhancement with arecoline and choline and impairment with scopolamine. Science 201: 274-276 (1978).

25. Stitcher, D. L., L. W. Harris, W. C. Heyl, and S. C. Alter. Effects of pyridostigmine and cholinolytics on cholinesterase and acetylcholine in Soman poisoned rats. Drug Chem Toxicol 1:355-362 (1978).

APPENDIX A

Titration Method for STM Measurement

The essential feature of the titration method is that it adjusts the retention interval in the delayed match-to-sample paradigm in such a way as to maintain the subject's success rate at a criterion level. This adjustment is accomplished by reducing the retention interval (for the next trial) after each error. The retention interval is increased after correct trials. Success rate is controlled either by the ratio of the increases to the decreases in retention interval (if it is changed on every trial) or (if increases and decreases are equal in size) by the ratio of the numbers of correct trials required for an increase to the number of incorrect trials required for a decrease. For example, if the retention interval is changed after every trial, and the increment is the same size as the decrement, the tendency is for the interval to vary around a value at which the animal is correct 50% of the time. This is the case because a success rate greater than 50% will cause the interval to shift upward, which will tend to lower the success rate. A success rate below 50% will cause the retention interval to shift downward, with a concomitant increase in success rate. Over a series of trials, the average interval will be the one at which the animal is able to achieve a 50% success rate. Thus, this average interval can be used as a direct measure of the animals' STM capability. When exposure to a drug changes this capability, the average interval will stabilize at a new level, but the success rate will remain near 50%. Other success rates can be achieved by adjustment of either the increment:decrement ratio (step sizes) or the errors per decrement:successes per increment ratio. For example, if either of these ratios is 1:2, the retention interval will equilibrate at a success rate of 66.7%, since a stable interval can only occur if two-thirds of the responses are correct.

For the present experiments, the criterion success rate was 75%. This was achieved by response-contingent increments and decrements of equal size, and a ratio of one error per decrement to three successes per increment. Thus, for each test session, the average retention interval was an unbiased estimate of the retention interval at which the animal could correctly recall the sample on 75% of the trials.

Figure A-1 is a flow chart that shows the sequence of events for each trial, the sources of the data that were collected, and the trial-to-trial sequential dependencies that determined the retention interval and the time-out delay for each trial. The time-out delay was increased in duration at short retention intervals to prevent animals from adopting a strategy of making runs of errors to reduce the retention interval to the minimum so that they would have more frequent opportunities to receive reward.

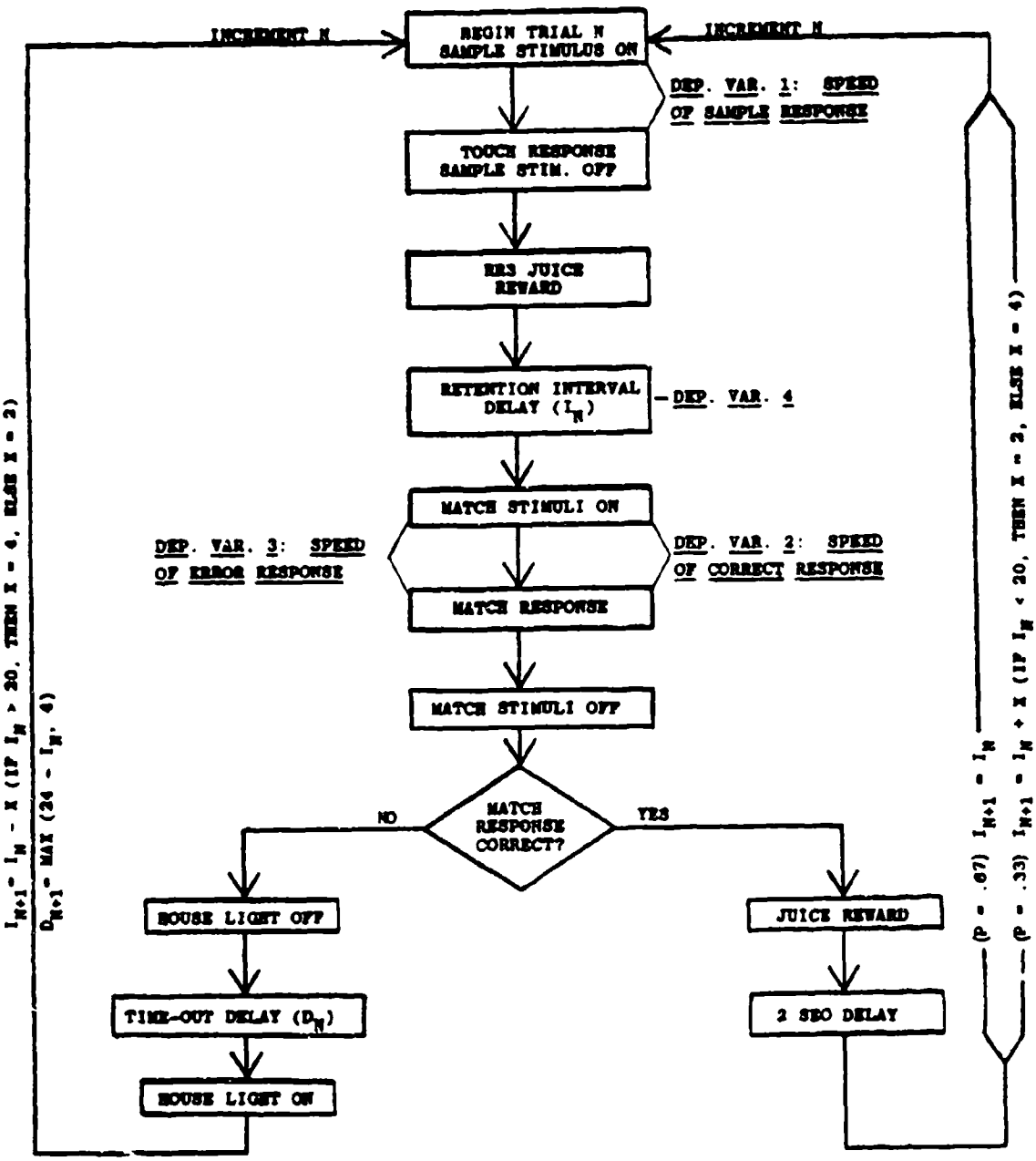

Figure A-1. Flow chart of contingencies in the titrated delayed match-to-sample procedure.

Appendix B

RESULTS OF ANALYSIS OF VARIANCE

TABLE B-1. PYRIDOSTIGMINE VS PHYSOSTIGMINE - EXPERIMENT I

A. Median Retention Interval (STM)

Source of Variance	Mean Squared Deviation	F Ratio	Degrees of Freedom	Tail Probability
Drug	37.10	2.40	1,5	0.182
Dose	8.57	1.15	3,15	0.361
Drug X Dose	45.25	1.41	3,15	0.279
Replication	86.30	4.23	1,5	0.095
Drug X Rep.	19.80	0.39	1,5	0.763
Dose X Rep.	7.37	1.49	3,15	0.277
Drug X Dose X Rep.	5.12	0.37	3,15	0.779

B. Speed of Sample Responses

Source of Variance	Mean Squared Deviation	F Ratio	Degrees of Freedom	Tail Probability
Drug	1.300	3.74	1,5	0.111
Dose	0.202	1.34	3,15	0.299
Drug X Dose	0.147	0.93	3,15	0.452
Replication	0.117	0.10	1,5	0.770
Drug X Rep.	0.402	2.94	1,5	0.147
Dose X Rep.	0.007	0.03	3,15	0.994
Drug X Dose X Rep.	0.186	1.34	3,15	0.299

C. Speed of Correct Match Responses

Source of Variance	Mean Squared Deviation	F Ratio	Degrees of Freedom	Tail Probability
Drug	0.064	8.99	1,5	0.030
Dose	0.073	1.53	3,15	0.021
Drug X Dose	0.107	4.37	3,15	0.021
Replication	0.084	1.19	1,5	0.325
Drug X Rep.	0.029	2.16	1,5	0.202
Dose X Rep.	0.019	0.70	3,15	0.565
Drug X Dose X Rep.	0.017	0.60	3,15	0.627

TABLE B-1. PYRIDOSTIGMINE VS PHYSOSTIGMINE - EXPERIMENT I (cont'd)

D. Speed of Error Match Responses

Source of Variance	Mean Squared Deviation	F Ratio	Degrees of Freedom	Tail Probability
Drug	0.174	4.30	1,5	0.093
Dose	0.009	0.28	3,15	0.838
Drug X Dose	0.116	3.11	3,15	0.058
Replication	0.001	0.02	1,5	0.899
Drug X Rep.	0.010	0.24	1,5	0.645
Dose X Rep.	0.009	0.28	3,15	0.838
Drug X Dose X Rep.	0.035	1.73	3,15	0.203

E. Number of Trials Completed under Physostigmine

Source of Variance	Mean Squared Deviation	F Ratio	Degrees of Freedom	Tail Probability
Dose	1305.25	4.36	3,15	0.021
Replication	163.35	0.42	1,5	0.547
Dose X Rep.	198.60	0.94	3,15	0.462

F. Number of Trials Completed under Pyridostigmine

Source of Variance	Mean Squared Deviation	F Ratio	Degrees of Freedom	Tail Probability
Dose	1140.39	2.09	3,15	0.159
Replication	5626.02	6.73	1,5	0.050
Dose X Rep.	473.56	0.73	3,15	0.529

TABLE B-2. ATROPINE VS METHYLATROPINE - EXPERIMENT II

A. Retention Interval (STM)

Source of Variance	Mean Squared Deviation	F Ratio	Degrees of Freedom	Tail Probability
Drug	28.286	1.75	1,5	0.244
Dose	13.894	0.48	3,15	0.704
Drug X Dose	5.639	0.33	3,15	0.802
Replication	150.225	7.64	1,5	0.040
Drug X Rep.	46.301	2.81	1,5	0.154
Dose X Rep.	5.478	0.27	3,15	0.846
Drug X Dose X Rep.	17.651	0.89	3,15	0.467

B. Speed of Sample Responses

Source of Variance	Mean Squared Deviation	F Ratio	Degrees of Freedom	Tail Probability
Drug	0.1291	0.46	1,5	0.526
Dose	0.3619	5.51	3,15	0.009
Drug X Dose	0.0450	0.17	3,15	0.913
Replication	0.2625	0.75	1,5	0.426
Drug X Rep.	0.4374	13.79	1,5	0.014
Dose X Rep.	0.1301	0.94	3,15	0.445
Drug X Dose X Rep.	0.0742	0.54	3,15	0.664

C. Speed of Correct Match Responses

Source of Variance	Mean Squared Deviation	F Ratio	Degrees of Freedom	Tail Probability
Drug	0.1592	5.36	1,5	0.068
Dose	0.0756	1.65	3,15	0.220
Drug X Dose	0.0287	1.10	3,15	0.378
Replication	0.1328	5.18	1,5	0.072
Drug X Rep.	0.0008	0.02	1,5	0.894
Dose X Rep.	0.0375	0.62	3,15	0.610
Drug X Dose X Rep.	0.0010	0.04	3,15	0.990

TABLE B-2. ATROPINE VS METHYLATROPINE - EXPERIMENT II (cont'd)

D. Speed of Error Match Responses

Source of Variance	Mean Squared Deviation	F Ratio	Degrees of Freedom	Tail Probability
Drug	0.1873	4.14	1,5	0.097
Dose	0.1212	4.02	3,15	0.028
Drug X Dose	0.0713	3.69	3,15	0.036
Replication	0.0228	1.54	1,5	0.270
Drug X Rep.	0.0070	0.46	1,5	0.529
Dose X Rep.	0.0113	0.28	3,15	0.839
Drug X Dose X Rep.	0.0068	0.44	3,15	0.728

E. Number of Trials Completed

Source of Variance	Mean Squared Deviation	F Ratio	Degrees of Freedom	Tail Probability
Drug	157.6	2.09	1,5	0.208
Dose	212.5	1.48	3,15	0.261
Drug X Dose	198.4	2.13	3,15	0.140
Replication	501.0	2.62	1,5	0.180
Drug X Rep.	0.5	0.02	1,5	0.905
Dose X Rep.	57.7	0.76	3,15	0.536
Drug X Dose X Rep.	100.4	1.39	3,15	0.284

TABLE B-3. ATROPINE VS PHYSOSTIGMINE - EXPERIMENT III

A. Retention Interval (STM)

Source of Variance	Mean Squared Deviation	F Ratio	Degrees of Freedom	Tail Probability
Atropine (A)	25.086	1.32	2,10	0.310
Physostigmine (P)	21.396	0.63	2,10	0.552
A X P	15.150	1.39	4,20	0.272

B. Speed of Sample Responses

Source of Variance	Mean Squared Deviation	F Ratio	Degrees of Freedom	Tail Probability
Atropine (A)	0.1248	0.84	2,10	0.459
Physostigmine (P)	0.1870	1.25	2,10	0.329
A X P	0.5974	10.99	4,20	0.0001

C. Speed of Correct Match Responses

Source of Variance	Mean Squared Deviation	F Ratio	Degrees of Freedom	Tail Probability
Atropine (A)	0.0261	1.71	2,10	0.230
Physostigmine (P)	0.0064	0.19	2,10	0.829
A X P	0.1857	18.35	4,20	0.0000

D. Speed of Error Match Responses

Source of Variance	Mean Squared Deviation	F Ratio	Degrees of Freedom	Tail Probability
Atropine (A)	0.0345	1.05	2,10	0.384
Physostigmine (P)	0.0147	0.26	2,10	0.779
A X P	0.1721	8.41	4,20	0.0004

E. Number of Trials Completed

Source of Variance	Mean Squared Deviation	F Ratio	Degrees of Freedom	Tail Probability
Atropine (A)	910.30	3.29	2,10	0.080
Physostigmine (P)	718.14	4.33	2,10	0.044
A X P	613.19	3.27	4,20	0.032